

CLAIMS:

1. A method of investigating single nucleotide polymorphisms in a sample of DNA, the method comprising contacting the DNA containing sample with at least one first set of primers, amplifying the DNA using those primers to give an amplified product, contacting at least a portion of the amplified product with at least one second set of primers, amplifying the DNA using those second set of primers to give a further amplified product and examining one or more characteristics of the further amplified product, one or more of the primers of the first set of primers including a locus specific portion and a further portion, the locus specific portion of one of those one or more of the primers annealing to one side of the SNP under investigation.
2. A method according to claim 1 in which one or more of the primers are provided with an SNP identifying portion, the SNP identifying portion being different for each different primer, the primer with an SNP identity portion which pairs to the SNP, annealing one side of the SNP.
3. A method according to claim 1 in which one or more of the second set of primers includes a second further portion, the second further portion being provided with a sequence equivalent to the sequence of the further portion of one or more of the primers of the first set which are provided with a locus specific portion and a further portion.
4. A method according to claim 1 in which the locus specific portion of the primers of the first set includes a sequence which matches the sequence of the locus sequence in the vicinity of the SNP under investigation, the match between the locus specific portion and sequence of the locus commencing at between one and ten bases to the respective sides of the SNP under investigation.
5. A method according to claim 1 in which the first set of primers includes a reverse primer and further includes a forward primer for each possible identity of the SNP under investigation.

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6. A method according to claim 1 in which the further portion of a primer is attached to the locus specific portion of the primer by an SNP related portion.
7. A method according to claim 6 in which the SNP identifying portion and SNP related portion of a primer have equivalent identity.
8. A method according to claim 1 in which the locus specific portion of the primers in a set are provided with identical sequences in each primer.
9. A method according to claim 1 in which the further portion includes a sequence which does not match the locus sequence on the locus' 3' side of the locus with sequence matching the locus specific portion of the primer.
10. A method according to claim 9 in which the sequence of the further portion does not anneal to a sequence of any published part of the entire DNA sequence of homo sapiens.
11. A method according to claim 1 in which the second set of primers includes a reverse primer and further includes a different forward primer for each potential identity of the SNP under investigation.
12. A method according to claim 1 in which the second further portion is attached to a second SNP identifying portion and / or an SNP repeat identifying portion.
13. A method according to claim 1 in which the second further portion includes a sequence which pairs to the sequence of the amplified product in the vicinity of the SNP identifying portion and / or SNP repeat related portion.

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14. A method according to claim 1 in which the sequence of the second further portion does not anneal to the sequence of any published part of the DNA sequence of homo sapiens.

15. A method according to claim 1 in which the SNP repeat identifying portion and / or second SNP identifying portion is a single nucleotide or two nucleotides, at least one of the nucleotides being identical to the SNP identifying portion and / or SNP related portion of a primer of the first set.

16. A method according to claim 1 in which a plurality of first sets of primers are provided to amplify a plurality of SNP loci, the amplification products resulting being of different lengths.

17. A method according to claim 1 in which one or more characteristics of the further amplified products are investigated by means of the presence and / or absence of a distinctive unit in the further amplified product.

18. A method according to claim 17 in which the distinctive unit is a dye, dye label, colour producing molecule, molecular beacon, emitter of radiation, characteristic isotope.

19. A method according to claim 17 in which the distinctive unit is provided at the 5' end of the forward primers, a different distinctive unit being provided for each forward primer of the second set.

20. A method according to claim 17 in which the distinctive unit is indicative of the nucleotide presence of the SNP.

21. A method according to claim 1 in which the further portion of at least one of the forward primers of the first set is different from the further portion of at least one of the other forward primers of the first set, at least in part.

22. A method according to claim 21 in which the forward primers are different from one another with respect to at least 25% of the nucleotides forming the further portion of the forward primers.
23. A method according to claim 21 in which the distinguishing portion is provided at an intermediate location within the sequence of the further portion.
24. A method according to claim 1 in which the further portion of one or more of the primers in the first set is provided with one or more portions which correspond with one or more portions in the further portion of one or more of the other primers of the first set.
25. A method according claim 21 in which the nucleotides of the further portion of the forward primers are equivalent to the nucleotides of the other forward primers, outside the distinguishing portion of the further portion.
26. A method according to claim 1 in which the first and second set of primers are present together and in which the concentration of the second set of primers is provided in a ratio relative to the concentration of the first set of primers of at least 5:1.
27. A method according to claim 1 in which the first and second set of primers are present together, the first set of primers is provided at a concentration of between 10 and 200nM and the second set is provided at a concentration of between 400 and 4000nM.
28. A method according to claim 1 in which the first and second set of primers are present together and the annealing temperature for at least some of the cycles of the amplification process is such that at least 80% of the second set of primers remain single stranded.
29. A method according to claim 28 in which the annealing temperature is so provided and used at least in cycles 3 to 30.

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30. A method according to claim 1 in which an annealing temperature is used in at least the last two cycles, the annealing temperature allowing at least 80% of the second set of primers to anneal.
31. A method according to claim 1 in which the annealing temperature is at least 72°C for cycles 3 to 30 of the amplification process.
32. A method according to claim 1 in which the annealing temperature for at least the last two cycles of the amplification process is 62°C or less.
33. A method according to claim 1 in which the amplification products of two or more first sets of primers and one or more second sets of primers are separated from one another using electrophoresis.
34. A method according to claim 1 in which the further amplified product is contacted with one or more components retained on a solid support, the one or more components having a sequence which anneals with at least part of the sequence of one of the further amplified products.
35. A method according to claim 34 in which the retained component anneals with the further amplified product up to the base before the base which is the SNP side.
36. A method according to claim 34 in which the retained component anneals to the further amplified product along the sequence corresponding to the locus specific portion and further portion of the further amplified product.
37. A method according to claim 1 in which a plurality of different retained components, preferably PCR products and / or oligonucleotides, are provided at discrete locations on a support, different retained components annealing to different further amplified products.

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38. A method according to claim 37 in which the retained component and annealed further amplified product are contacted with one or more further components to introduce a distinctive unit.
39. A method according to claim 37 in which the retained component and annealed further amplified product are contacted with one or more additional components, the one or more additional component being one or more further oligonucleotides which include a distinctive unit.
40. A method according to claim 39 in which the end base of the further oligonucleotide is one of the four possible identities for the SNP.
41. A method according to claim 1 in which the further amplified product includes an attachment unit and the attachment unit facilitates attachment of the further amplified product to a solid support.
42. A method according to claim 41 in which the attached further amplified product is contacted with one or more probes having different sequences from one another, at least in part.
43. A method according to claim 41 in which each probe has a common sequence portion to each other, the common sequence portion corresponding in sequence to the locus specific portion of the further amplified product.
44. A method according to claim 41 in which the probes incorporate at least one different sequence portion compared with one another, the different portion of at least one of the probes corresponding to the further primer portion sequence of the further amplified product.

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45. A method according to claim 41 in which contact of the probes with the further amplified product results in hybridisation of one of the probes to the further amplified product, each probe having a distinctive unit relative to one another.

46. A plurality of primers for investigating single nucleotide polymorphisms in the sample of DNA, the plurality of primers comprising two or more primers of a first set of primers and / or two or more primers of a second set of primers, one or more of the primers of the first set of primers having a locus specific portion and a further portion.